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Nephro-Protection Potentials of N-Hexane Extract of *Anonna Muricata* Leaves on Isoniazid Induced Kidney Damage in Wistar Rat

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Abstract: The objective of this research was to assess the protective effect of n-hexane extract of *Annona muricata* on kidney damage induced by isoniazid in Wistar rats. The study involved measuring serum levels of urea, creatinine, sodium, potassium, chloride, and bicarbonate, along with antioxidant activities in kidney tissue, including superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and malondialdehyde (MDA). A completely randomized block design (CRBD) was employed, with 25 Wistar rats divided into five groups. Groups 1 and 2 served as the normal and positive controls, respectively, while groups 3, 4, and 5 were pretreated with varying doses of *Annona muricata* extract (200 mg/kg and 400 mg/kg) or vitamin E. After 21 days of treatment, kidney damage was induced by administering isoniazid (50 mg/kg) to all groups except the normal control. Blood samples were collected for kidney function analysis, and kidney tissues were homogenized for antioxidant assays. The results indicated that isoniazid significantly increased serum levels of urea, creatinine, sodium, potassium, chloride, and MDA, while decreasing levels of SOD, CAT, GSH, and bicarbonate. Treatment with *Annona muricata* extract significantly improved these parameters, suggesting that the extract possesses antioxidant properties that protect the kidneys from isoniazid-induced toxicity in Wistar rats.

Keywords: treatment, extract, protection, antioxidant and kidney

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Introduction

Isoniazid is an effective antitubercular agent but is associated with several side effects, including liver and kidney damage. The nephrotoxic effects of isoniazid can lead to elevated serum creatinine and urea levels, indicating impaired kidney function. Annona muricata, or soursop, is a tropical fruit known for its antioxidant, anti-inflammatory, and hepatoprotective properties (Erukainure *et al.*, 2015). This study investigates the protective role of *Annona muricata* extracts against isoniazid-induced kidney damage in Wistar rats. *Annona muricata*, commonly known as soursop or graviola, is a tropical fruit-bearing tree native to the Caribbean, Central America, and South America. It is well-regarded for its medicinal properties and is used in traditional medicine for various health conditions (Fabricant & Farnsworth, 2001). Several phytochemicals have been identified in *Annona muricata*, contributing to its therapeutic potential, flavonoids, tannins, vitamin C, vitamin B and riboflavin and minerals such as potassium, magnesium, and iron which contribute to its nutritional value and health-promoting properties. These phytochemicals work synergistically to exert various pharmacological effects, including anticancer, antioxidant, antimicrobial, anti-inflammatory and antiparasitic activities. (Ramos *et al.*, 2022).

Isoniazid, commonly abbreviated as INH, is a potent medication primarily used in the treatment of tuberculosis (TB) and latent tuberculosis infection (LTBI). It is often included as a key component of first-line TB therapy due to its effectiveness in killing Mycobacterium tuberculosis, the bacterium responsible for TB (Mohammed *et al.*, 2018). The primary mode of action of isoniazid involves inhibiting the synthesis of mycolic acids, essential components of the bacterial cell wall. By disrupting this process, isoniazid effectively kills or slows the growth of M. tuberculosis, leading to the elimination of the infection. Despite its efficacy in treating TB, isoniazid is associated with various adverse effects and toxicities (Azimi and Khodaie, 2017). Medicinal plants are rich in phytochemicals which confer on them the ability to provide healing and protection to the body. Phytochemicals protect

plants against diseases caused by fungi, bacteria, and viruses, as well as deter insects and other animals. They can be found in various plant parts such as leaves, fruits, flowers, seeds, roots, stems, barks, and rhizomes (Sofowora *et al.*, 2008). These natural chemicals are considered non-essential for human survival as they do not provide nutrition. However, they have been found to protect humans from fatal diseases and offer numerous health benefits. (Goossens *et al*., 2020). Some of the common side effects include hepatotoxicity (liver damage) which range from mild elevations in liver enzymes to severe hepatitis or even fulminant hepatic failure. Others are peripheral neuropathy, nephritis and gastrointestinal disturbances (Anosike & Obidoa, 2018).

Materials and Methods

Study area

The administration of treatment to the animal subjects was carried out in the animal the Department of Biochemistry, Niger Delta University. Similarly, the biochemical analysis took place at the Chemical Pathology Laboratory of the Niger Delta University Teaching Hospital, Okolobiri, Bayelsa State. Okolobiri is a rural village situated in the Yenagoa Local Government Area of Bayelsa State. With a population of over 20,000, it is essentially a riverine town located at 4.88540N and 6.07840E geographical coordinates. Okolobiri is a fast-developing suburb and is host several multinational oil companies and has been plagued by oil pollution for decades. The common vocation of natives and residents of Okolobiri are mainly farming, fishing, petty traders, company workers and civil servants (Ikimi and Addy, 2024).

Collection and identification of plant sample

Fresh leaves *Annona muricata* were collected from the premises of College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State. The plant was identified by Professor Kola Ajibesin of the Department of Pharmacognosy, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State.

Preparation of plant extract

The plant leaves were collected in large quantity and left under shade at room temperature for 2 weeks to dry. Afterwards, they were ground into powder. Thereafter, 247g of the powder was soaked in 1.5litres of n-hexane and allowed to stand for 48hours. The extract was collected, filtered and concentrated under increased pressure using a rotary evaporator at 60°C and 40 rpm

Animal specimen and study population

Twenty-five healthy male wistar albino rats weighing between 200g and 300g were used for this study. The animals were procured from the animal house of the University of Port Harcourt. The rats were kept in standard animal cages and acclimatized in the animal house, in the Department of Pharmacology, Niger Delta University, for 14 days and allowed free access to pelleted chicken feed and water. Mead's resource equation was utilized for the calculation of the sample size (Kirkwood and Robert, 2010). The equation is stated and the components defined. E = N – B - T, where: N is the total number of individuals or units in the study (minus 1). B is the blocking component, representing environmental effects allowed for in the design (minus 1). T is the treatment component, corresponding to the number of treatment groups (including control group) being used, or the number of questions being asked (minus 1). E is the degrees of freedom of the error component, and should be somewhere between 10 and 20. The study constituted of five groups (T = 4), with 5 animals per group, making 25 animals in total (N = 24), without any further stratification (B = 0), then E would equal 20, indicating that the sample size is very suitable for the research

Ethical clearance

Ethical clearance was obtained from the animal research ethics committee of the Department of Biochemistry, Niger Delta University, Bayelsa State. The Animal Welfare Act of 1985 of the United States of America for research and Institutional Animal Care and Use Committee (IACUC) protocols were stringently adhered to (Benjamin & Jean, 2016).

Experimental design

Completely randomized block design (CRBD) was used for this study. The animals were randomly grouped into 5 groups of 5 rats each in a standard rat cage and were treated as follows:

Group 1 (Normal control): were fed with only pelleted chicken feed and distilled water.

Group 2 (Positive control): were administered isoniazid (50 mg/kg) daily for 21 days.

Group 3 (Test group 1): were administered 200 mg/kg of plant extract + 50 mg/kg isoniazid for 21 days.

Group 4 (Test group 2): were administered 400mg/kg of plant extract + 50 mg/kg isoniazid for 21 days.

Group 5 (Standard control): were administered Vitamin E (200 mg/kg) + 50 mg/kg isoniazid for 21 days.

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All groups were fed with pelleted chicken feed and distilled water *ad libitum*.

Selection criteria

Rats used were apparently healthy and active as confirmed and approved by a veterinary doctor. Rats showing signs and symptoms of illness were excluded from the research. Also excluded were rats with any form of derangements. Contaminated blood samples were rejected.

Collection and preparation of blood and tissue samples

Blood was collected via cardiac puncture into plain bottles and allowed to stand for 30mins for coagulation to take place so as to obtain only the serum. Afterwards, the blood samples were centrifuged for 10mins at 2000 rpm and the supernatant was collected for the analysis. A portion of the kidney was also collected to prepare the homogenate for the antioxidant assays (Sule *et al.*, 2017; Ness, 1999).

Analysis of samples

Serum urea, creatinine, sodium, potassium and chloride were determined by spectrophotometric methods using Randox kits, as reported by Ikimi *et al.*, 2023 and Ikimi *et al.*, 2024. Serum bicarbonate was determined using an ion selective electrode (ISE) analyzer method (analyzer ISE 4000) as stated by Bolarin and Azinger (2010). SOD activity was determined by the method of Xin *et al.*, (1991) as contained in Randox commercial kit leaflet; the method of Habig *et al.*, (1974) was followed in estimating the level of reduced glutathione (GSH); catalase activity was determined according to the method of Aebi, (1983); lipid peroxidation analyses was carried out by determining the concentration of MDA formed using the method of Varshney and Kale (1990), as modified and reported in Agoro *et al.* (2017) and Agoro *et al.* (2019)

Statistical analysis

Results were analysed statistically using One-way analysis of variance (ANOVA) under Turkey Kramer Multiple Comparison Test and Statistical Package for Social Sciences (SPSS) version 20. Values are expressed as mean standard deviation (SD) and were considered statistically significant at $p< 0.05$.

Results

Table 1 shows that pretreatment with *Annona muricata* 400 mg/kg body weight (test group 2) caused a significant ($p < 0.05$) decrease in the rate of weight gain (22%) when compared with normal (40%) and positive (38.8%) controls.

Table 1: Effect of isoniazid and *Annona muricata* on the mean body weight (g) of wistar rats

Experimental group	Mean weight before treatment (g)	Mean weight after treatment (g)	% Mean weight change
Normal control	158.6±2.70	198±11.02	40[a]
Positive control	159.8±3.11	198.6±8.70	38.8[a]
Test Group 1	160.4±3.05	201.8±8.26	41.4[a]
Test Group 2	159.8±5.07	181.8±36.06	22[b]
Standard control	162.4±4.04	203.8±3.77	41.4[a]

Data are expressed as the mean ± SD (n = 5). Means within the same column carrying same superscripts are not significantly ($p < 0.05$) different.

Table 2 shows that isoniazid administration caused a significant increase ($p<0.05$) in serum concentrations of urea (102.76±4.36), creatinine (3.14±0.47), bicarbonate (22.99±1.34), potassium (3.06±0.15), sodium (111.98±5.7) and chloride (59.22±5.57) (positive control) relative to normal control. However, pretreatment with *Annona muricata* at doses of 200 mg/kg body weight and 400 mg/kg body weight showed a significant ($p<0.05$) dose dependent decrease in serum concentrations of urea (80.47±3.51 and 76.01±2.23), creatinine (1.75±0.34 and1.07±0.46), bicarbonate (30.85±1.79 and 36.83±2.92), potassium (2.58±0.25 and 2.06±0.08), sodium (87.49±1.61 and 78.79±2.21) and chloride (48.39±2.18 and 39.27±2.18) respectively, relative to other isoniazid treated groups.

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Table 2: Effects of *Annona muricata* on the levels of kidney parameters in isoniazid induced kidney damage in wistar rats

Experimental group	Urea (mg/dL)	Creatinine (mg/dL)	Bicarbonate (mmol/L)	Potassium (mEq/L)	Sodium (mEq/L)	Chloride (mEq/L)
Normal control	62.40±1.22[a]	0.67±0.01[a]	40.51±0.06[a]	1.49±0.07[a]	68.91±1.60[a]	23.35±2.34[a]
Positive control	102.76±4.36[b]	3.14±0.47[b]	22.99±1.34[b]	3.06±0.151[b]	111.98±5.7[b]	59.22±5.57[b]
Test group 1	80.47±3.51[c]	1.75±0.34[c]	30.85±1.79[c]	2.58±0.25 [b]	87.49±1.61[c]	48.39±2.18[c]
Test group 2	76.01±2.23[c]	1.07±0.46[d]	36.83±2.92[d]	2.06±0.08[c]	78.79±2.21[d]	39.27±2.18[d]
Standard control	69.59±1.47 [d]	0.93±0.37[d]	39.02±1.77[d]	1.88±0.06[c]	77.29±0.06[d]	30.94±2.86[e]

Data are expressed as the mean ± SD (n = 5). Means within the same column carrying same superscripts are not significantly ($p < 0.05$) different.

Table 3 shows that isoniazid administration caused a significant decrease ($p<0.05$) in kidney SOD (2.78±0.12), CAT (2.26±0.22) and GSH (2.29±0.41) activities (positive control) and a significant increase ($p<0.05$) in kidney MDA concentration (6.00±0.14) relative to normal controls. However, treatment with *Annona muricata* at doses of 200 mg/kg body weight and 400 mg/kg body weight caused a significant ($p<0.05$) dose dependent elevation of kidney activities of SOD (4.89±0.08 and 5.80±0.20), CAT (3.95±0.18 and 4.72±0.24) and GSH (4.08±0.16 and 4.78±0.17) and a significant decrease in the kidney MDA (4.01±0.22 and 3.39±0.37) concentration respectively relative to other isoniazid treated groups.

Table 3: Antioxidant effects of *Annona muricata* on isoniazid induced kidney damage in wistar rats

Experimental group	SOD (U/mg protein)	Catalase (U/mg protein)	GSH (U/mg protein)	MDA (U/mg protein)
Normal control	8.09±0.50a	7.15±0.16[a]	7.12±0.10[a]	2.00±0.07[a]
Positive Control	2.78±0.12[b]	2.26±0.22[b]	2.29±0.41[a]	6.00±0.14[b]
Test Group 1	4.89±0.08[c]	3.95±0.18[c]	4.08±0.16[b]	4.01±0.22[c]
Test Group 2	5.80±0.20[d]	4.72±0.24[d]	4.78±0.17[c]	3.39±0.37[c]
Standard control	5.85±0.23[d]	6.16±0.49[e]	4.72±0.55[c]	2.84±0.16[d]

Data are expressed as the mean ± SD (n = 5). Means within the same column carrying same superscripts are not significantly ($p < 0.05$) different.

Discussion

Medicinal plants have long been utilized in various cultures as a primary source of healthcare, with many studied for their therapeutic potential, including anticancer, antidiabetic, antimicrobial, and antiviral properties (Mohammed, 2019; Opara *et al.*, 2021). This investigation specifically focused on examining the influence of n-hexane extract of *Annona muricata* leaf extract on kidney injury provoked by high doses of isoniazid in wistar rats. The findings indicated that pre-administration of *Annona muricata* extract (at doses of 200 mg/kg body weight and 400 mg/kg body weight) resulted in a significant reduction in weight gain compared to the normal and positive control groups, suggesting potential weight management properties of the extract. The kidneys play a vital role in eliminating waste products and toxins like urea, creatinine, and a small number of conjugated substances (Hoilat & John, 2022). Results (table 2) demonstrated that isoniazid exposure led to a substantial elevation in serum concentrations of

urea, creatinine, chloride, sodium, and potassium, while bicarbonate levels decreased compared to the normal control group, indicating renal damage and dysfunction. However, pretreatment with n-hexane extract of *Annona muricata* (at doses of 200 mg/kg and 400 mg/kg body weight respectively) significantly mitigated the elevation of urea, creatinine, chloride, sodium, and potassium concentrations and increased bicarbonate levels when compared with the positive control. Treatment with vitamin E also exhibited significant reductions in these serum parameters that is comparable to the higher dose of *Annona muricata* (group 4), indicating its potential protective effects on the kidneys. Several studies have explored the potential effects of *Annona muricata*. A study by Mohammed and Abbas (2016) investigated the effects of *Annona muricata* on ischemia-reperfusion injury in rats. They found that the extract improved cardiac antioxidant enzyme activity and reduced tissue damage. This further supports the current study's findings regarding the protective effects of *Annona muricata*. Isoniazid is a widely used antibiotic for the treatment of tuberculosis. However, it has been known to cause hepatotoxicity and oxidative stress as side effects **(Nwankpa *et al.*, 2017).**

Antioxidants play a crucial role in preventing cellular damage caused by free radicals generated during various metabolic processes (Sharifi-Rad *et al.*, 2020). The study also assessed antioxidant parameters, revealing that isoniazid intoxication significantly increased kidney MDA concentration while reducing the activities of SOD, CAT and GSH enzymes, indicating heightened lipid peroxidation and compromised antioxidant defense mechanisms. Treatment with *Annona muricata* extract and vitamin E reversed these effects, suggesting antioxidant properties of the extract. These findings align with that of Umoren *et al.*, (2023), who demonstrated that *Annona muricata* leaf extract prevented hepato-renal toxicity in male wistar rats by enhancing in vivo antioxidant defenses. Isoniazid induced kidney damage in rats, as evidenced by decreased SOD and CAT activity, GSH and increased MDA levels. The findings of this study align with that of Mohammed *et al.*, (2019) and Mohamed *et al.*, (2017), who reported similar findings in various animal models. This study suggests that *Annona muricata* may offer some protection against isoniazid-induced oxidative stress. The partial restoration of SOD levels and the prevention of MDA increase in groups 3 and 4 indicate a potential antioxidant effect. This is in line with the findings of Abdul-Wahab *et al.*, (2018) and Ilango *et al.*, (2021) who reported that *Annona muricata* extract exhibited free radical scavenging activity in vitro. However, the effect of *Annona muricata* was not as pronounced as vitamin E (group 5), which significantly increased SOD levels compared to all other groups. This is unsurprising as vitamin E is a well-established antioxidant with numerous scientific studies supporting its efficacy **(Rychter *et al.*, 2022).**

Conclusion

This result of this study suggests that the n-hexane extract of *Annona muricata* may contain antioxidant compounds with nephroprotective properties in rats induced with isoniazid, and these protective effects were dose-dependent. Further investigations are crucial to unravel the specific mechanisms through which *Annona muricata* provides kidney protection.

Conflict of interests

The authors declare no conflict of interests

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